

Purification and characterization of an anticoagulant from the salivary glands of the ixodid tick *Rhipicephalus appendiculatus*

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Abstract:

An anticoagulant isolated from salivary gland extracts of the ixodid tick *Rhipicephalus appendiculatus* was purified by gel filtration on Sephadex G-100, ion exchange on DEAE-cellulose, aprotinin-Sepharose, and by high-pressure-liquid size-exclusion chromatography. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that the anticoagulant activity was associated with a protein of an apparent Mr of 65 kDa. The purified molecule had a pI in the range of 8.0-8.5 on chromatofocusing and was stable over a wide pH range, but was heat labile and susceptible to inactivation by trypsin and reductive alkylation. The anticoagulant did not inhibit thrombin-initiated fibrin formation and had no detectable fibrino(geno)lytic or phospholipase-like activities. Although it inhibited factor Xa-induced clotting of bovine plasma, it did not affect the amidase activity of factor Xa toward a synthetic substrate, suggesting that the anticoagulant acts at a site distinct from the active site of factor Xa or on other components of the prothrombinase complex